Influence of the Linker in Bispyridium Compounds on the Inhibition of Human Choline Kinase

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Studies have been aimed to establish the structure−activity relationship that define choline kinase (ChoK) inhibitory potency and antiproliferative activity of a set of 25 bispyridinium compounds with electron-releasing groups at position 4. Here we report that, according to their inhibitory activities against human ChoK, the enzymatic inhibitory potency is closely related to the size of the linker, the 3,3′-biphenyl moiety being the most suitable. The *N*-methylanilino and its derivatives, 4-chloro-*N*-methylanilino and 3,5-dichloro-*N*-methylanilino, render higher ChoK inhibitory and antiproliferative activities against the HT-29 human colon cancer cell line.

Introduction

The discovery of oncogenes and tumor suppressor genes has been a great advance to foster the fight against cancer.[1−6] In particular, elucidation of the properties and functions of the products of some of the most common human oncogenes has been essential for the design of novel antitumor strategies. Once activated, oncogenes trigger a series of biochemical pathways, such as protein phosphorylation cascades and alteration of phospholipid metabolites, that entail an unrestricted cellular proliferation.[1−6] One of the most relevant alterations recently found associated to oncogenic transformation implies an increase of intracellular phosphorylcholine (*P*Cho) as a consequence of choline kinase (ChoK) activation.[7−9] This has been the basis for the use of inhibition of ChoK as a novel antitumor strategy[10−12] and have accomplished a rigorous process of molecular variation and design of potent ChoK inhibitors using hemicholinium-3 (HC-3) as a model.[13,14] A high number of symmetrical bispyridinium compounds have been designed, synthesized, characterized, and biologically assessed.[15−20] These compounds fall into two structural categories: (a) acyclic bis- and tris-pyridinium compounds, and (b) bispyridinium cyclophanes, which can be considered as rigid analogues of the previous biscationic ones. The ChoK inhibitory potency is notably increased by closing the stuctures[20] and by the introduction of a third cationic head.[18] On the contrary, both modifications produce a decrease in the antiproliferative capacity due to the low lipophilicity of the compounds. Previous QSAR studies demonstrated that the presence of an electron-releasing group at position 4 of the pyridinium ring favors both the ChoK inhibitory and antiproliferative activities.[15] The enzyme inhibition was explained on the basis of other processes which are important for the strength of the drug−ChoK interaction such as the desolvation of the compounds.[16]

Here, we further explore the structure−activity relationship of a series of newly designed ChoK inhibitors and study the structural parameters that define both the ChoK inhibitory and antiproliferative activities. The synthesis of a new set of compounds is reported based on molecular variations of the biscationic acyclic compounds. The aim of this study specifically focuses on the expected effect on the biological activities by a variation in the linker that connects the pyridinium cations having electron-releasing groups at their position 4.

Previous studies demonstrate that bisquaternized protein kinase C (PKC) inhibitory compounds notably increase their activities on augmenting the linker length.[21] Since our research seeks for a ChoK selective inhibition, the synthesis of five families of final compounds is suggested with linkers that decrease the distance between both cationic heads. The model used has been the most active antiproliferative biscationic acyclic compound so far reported, having a bibenzyl linker and in which R is a *N*-methylanilino moiety, with an IC_{50} against human ChoK of 7.2 μM and an antiproliferative activity (IC_{50} HT-29) equal to 0.18 μM.[17]

Figure 1 shows the general structures of the synthesized compounds. It can be noted that the separation between the cationic heads increases on going from the *trans*-1,2-cyclopropane to the 4,4′-biphenyl spacers. The linker *cis*-1,2-cyclopropane was not used because of the extreme closeness of the positive charges which would make the synthesis of their analogues more troublesome.

Chemistry

Twenty five final compounds, included in five families, have been synthesized (Table 1). They are biscationic compounds that consist of a linker and two cationic heads which are 4-substituted pyridinium rings with tertiary cyclic and acyclic amino groups.

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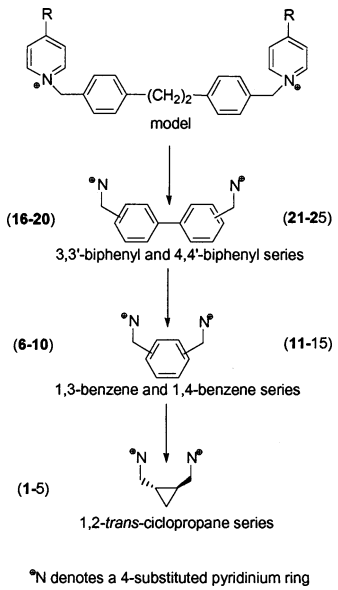


Figure 1. Structures of the five families of the target molecules that show a decrease in length of the spacer. The structures generated in this study are shown

Five different types of linkers have been used; two of them, 1,3-bis(bromomethyl)benzene and 1,4-bis(bromomethyl)benzene, are commercially available. *trans*-1,2-Bis(bromomethyl)cyclopropane was obtained by reduction of the commercial diester with lithium aluminum hydride[22] to give the corresponding diol, with subsequent substitution of the two oxygenated functions with phosphorus tribromide.[23] The 3,3′-bis(bromomethyl)-biphenyl linker is obtained through a radical benzylic halogenation of the commercially available 3,3′-dimethylbiphenyl with *N*-bromosuccinimide (NBS) and benzoyl peroxide as catalyst.[24] Finally, the synthesis of 4,4′-bis-(bromomethyl)biphenyl was carried out by reaction between formaldehyde (or its polymers) and hydrogen bromide in the presence of *o*-phosphoric acid.[25,26]

Two of the 4-substituted pyridines are commercially available: 4-(*N*,*N*-dimethylamino)pyridine and 4-(pyrrolidino)piridine; the rest have been synthesized from 4-chloropyridine by a previously reported method.[18]

The synthesis of the acyclic final compounds was carried out by heating of the corresponding bromide and heterocyclic derivatives (in a molar ratio 1:2) using butanone as solvent.[17] The reaction was carried out in a sealed tube and at a temperature of 100 °C.

Biological Testing

Compounds **1**−**25** were tested in an ex vivo system using recombinant human ChoK as a target as previously reported.[9,12] This assay allowed us to evaluate the inhibitory potency of the compounds toward ChoK, without the possible interference due to permeability through membranes. The HT-29 cell line was established from a human colon adenocarcinoma,[27] and it is considered an appropriate system for the search of new antitumor drugs. The effects on cell proliferation by all the compounds were investigated on the HT-29 cell line (in vitro assay) by the crystal violet method as previously reported.[9,12,28] IC_{50} values were obtained from nonlinear least-squares fit of the Hill equation to the data. The activity in the in vitro assay reflects the pharmacodynamic properties of the compounds, but it can be also a measurement of their affinity for the enzyme.

Results and Discussion

The biological results of the final compounds **1**−**25** are shown in Table 1.

Cyclopropane Derivatives. In this series only one compound shows activity on the recombinant enzyme, and only two compounds had activity against the HT-29 cancer cell line. Therefore, it can be stated that the fact of using a small linker such as 1,2-*trans*-cyclopropane leads to compounds with a lesser ChoK inhibitory activity. The utilization of such a linker implies a fall in the volume, in its lipophilicity, and in the distance that keeps the cationic heads apart, which results in a decrease of the enzymatic inhibitory potency.

The most active compound as an antiproliferative agent in this series is **5** with an IC_{50} of 7.5 μM on HT-29, although it has no ChoK inhibitory activity. On the other hand, **4**, which is capable of weakly inhibiting the enzyme (IC_{50} ChoK = 48.2 μM), shows worse antiproliferative activity than **5** (IC_{50} HT-29 = 24.6 μM). The fact that these compounds are not good ChoK inhibitors but have antiproliferative activity suggests that they could act somewhere within the mitogenic signaling pathway.

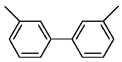
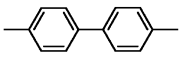
1,3-Benzene and 1,4-Benzene Derivatives. These compounds are more active than those of the previous series. This suggests that an increase in the linker volume improves its biological activity determined as both the inhibition of the enzyme and the inhibition of cellular proliferation.

If the ChoK inhibitory values of 1,3-benzene derivatives are compared with those of the 1,4-benzene ones, it is observed that the second group presents better values of the enzymatic activity than their corresponding isomers derived from 1,3-benzene. A possible explanation is that the inhibition against the enzyme is also influenced by the distance between cationic heads in addition to lipophilicity, since these two linkers have a nearly identical lipophilicity, being different only in the distance between the cationic heads, lower in the former than in the latter: the longer the distance between the cationic heads the better the ChoK inhibition.

3,3′-Biphenyl and 4,4′-Biphenyl Derivatives. These 10 compounds show better ChoK inhibitory activities than those shown by the three previous series, an indication of the importance that this linker plays. Compound **18** shows a submicromolar ChoK inhibitory activity (IC_{50} ChoK = 0.43 μM). From all the final compounds obtained in this study, **24** and **25** show higher values for antiproliferative activities with IC_{50} values against HT-29 of 0.49 and 0.65 μM, respectively.

But in this case, in contrast to 1,3- and 1,4-benzene derivatives, the ChoK inhibitory activity is higher when the distance between the cationic heads is decreased: that is, the ChoK IC_{50} values obtained for the 3,3′-biphenyl derivatives are better than the ones obtained for their corresponding isomers derived from 4,4′-biphenyl. This may imply that the increase in the distance between the cationic heads augments the ChoK inhibitory activity up to an optimum value that corresponds to the 3,3′-biphenyl linker. On surpassing this distance (this is the case when the 4,4′-biphenyl moiety

Table 1. IC_{50} ChoK and HT-29 Values for the Biscationic Compounds

Comp.	Linker	R	IC_{50} ChoK (μM)	IC_{50} HT-29 (μM)
1		pyrrolidino	>100	>100
2		piperidino	98	99
3		*N*-methylanilino	>100	>100
4		4-chloro-*N*-methylanilino	48.2	24.3
5		3,5-dichloro-*N*-methylanilino	>100	7.5
6		pyrrolidino	84.2	50
7		piperidino	52.8	43
8		*N*-methylanilino	37.5	5.1
9		4-chloro-*N*-methylanilino	7.2	3.3
10		3,5-dichloro-*N*-methylanilino	8.4	4
11		pyrrolidino	24	>100
12		piperidino	37.7	>100
13		*N*-methylanilino	15	6.81
14		4-chloro-*N*-methylanilino	2.9	3.5
15		3,5-dichloro-*N*-methylanilino	9.7	2.9
16		pyrrolidino	5.8	2.6
17		piperidino	1.9	1.6
18		*N*-methylanilino	0.40	2.2
19		4-chloro-*N*-methylanilino	1.5	1.9
20		3,5-dichloro-*N*-methylanilino	2.6	1.8
21		pyrrolidino	11.1	6.1
22		piperidino	8.8	4.2
23		*N*-methylanilino	4.3	2
24		4-chloro-*N*-methylanilino	1.8	0.49
25		3,5-dichloro-*N*-methylanilino	3.4	0.65

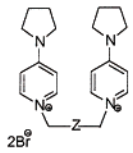
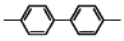
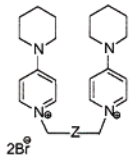
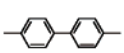
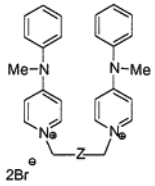
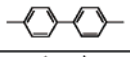
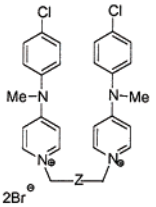
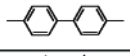
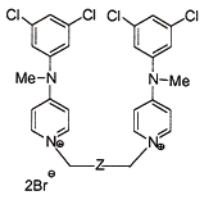
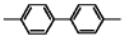
is used) the activity decreases again. The relevance of this finding is discussed below.

The Linker Influence. With the aim of clarifying how the nature of the linker affects ChoK inhibitory activity, the activities of the compounds are presented, differing each other only in the linker that keeps the cationic heads apart (Table 2).

It is observed that the ChoK inhibitory activity increases in a progressive way as the distance between the cationic heads is increased to an optimum value, which in all the cases is the corresponding distance to the 3,3′-biphenyl spacer. Once such a distance is exceeded (when the 4,4′-biphenyl spacer is used), the enzymatic inhibitory potency drops again. (The only case in which this does not hold is that of compounds **10** and **15** (Table 2) which are practically equipotent.)

The relationship between the antiproliferative activity and the distance between the cationic heads is not

Table 2. IC_{50} ChoK Values for the Final Compounds Grouped by the Substituent at Position 4 of the Pyridinium Ring

General Structure	Comp.	Z	IC_{50} ChoK (μM)
	1		>100
	6		84.2
	11		24
	16		5.8
	21		11.1
	2		98
	7		52.8
	12		37.7
	17		1.9
	22		8.8
	3		>100
	8		37.5
	13		15
	18		0.43
	23		4.3
	4		48.2
	9		7.2
	14		2.9
	19		1.5
	24		1.8
	5		>100
	10		8.4[a]
	15		9.7[a]
	20		2.6
	25		3.4

Increase in the distance between cationic heads

Increase in ChoK Inhibition

[a] Compounds **10** and **15** are practically equipotent.

straighforward (see Table 1). This may be logical because some other factors may influence the in vitro activity, such as cell permeability.

Influence of the Pyridine R Substituent. The *N*-methylanilino group and its derivatives are the substituents at position 4 of the pyridinium ring that give rise to compounds with better ChoK inhibitory and antiproliferative activities. As can be noted in the biological results in all the series discussed (Table 1), the ChoK inhibitory activity gradually increases from the dimethylamino to the 4-chloro-*N*-methylanilino moieties (except in the case of 3,3′-biphenyl derivatives in which compound **18** is the most active one with the *N*-metilanilino substituent), but the 3,5-dichloro-*N*-methylanilino substituent provokes a decrease in the activity in relation to the latter. This effect is not so clear for the antiproliferative activity.

Correaltion of Inhibition of ChoK and Cytotoxicity. An increase in ChoK inhibitory activity is associated with an increase in the antiproliferative activity toward HT29 cells. This is exemplified in Figure 2, where the correlation of both activites are represented for compounds **21** to **25**. Thus, these results strongly support the relevance of ChoK inhibition for cytotoxicity of the studied bispyridium compounds.

Conclusions

According to the data for the ChoK inhibitory activity found for the acyclic biscationic pyridophanes, it can be suggested that the enzymatic inhibitory potency is

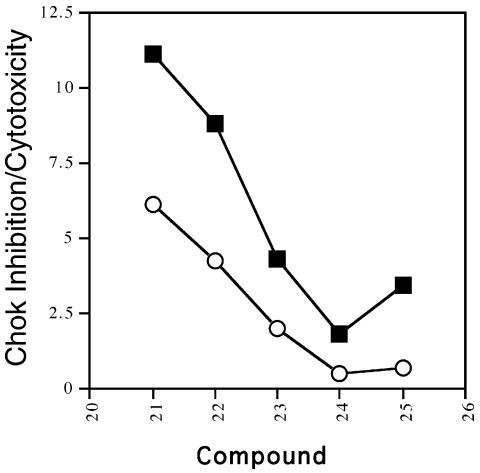


Figure 2. Relationship between ex vivo ChoK inhibition and in vitro cytotoxicity activity of compounds **21** to **25**. Activities for each compound in this group is represented as the micromolar concentrations at which the IC_{50} is achieved.for either ChoK inhibition ex vivo (■) or the antiproliferative (cytotoxic) activity against HT29 cells (○).

closely related to the size of the spacer. Thus, compounds that show the higher inhibitory activity against the isolated enzyme are those that contain the 3,3′-biphenyl spacer. The relationship between the antiproliferative activity and the distance between the cationic heads is not so exact. This is reasonable because in the measure of the activity against the HT-29 cell line, in addition to the interaction with ChoK, some other factors may bear an influence.

From the six electron-releasing substituents studied, *N*-methylanilino and its derivatives 4-chloro-*N*-methylanilino and 3,5-dichloro-*N*-methylanilino are the groups that, located at position 4 of the pyridinium fragment, lead to higher ChoK inhibitory and antiproliferative activities against the HT-29 human colon cancer cell line.

Experimental Section

(a) Chemistry. For general procedures see ref 20. All compounds were dried at 40 °C and 0.1 mmHg for 24 h, but many held on tenaciously to water and/or ethanol which appear to be solvates. *trans*-1,2-Bis(hydroxymethyl)cyclopropane,[22] *trans*-1,2-bis(bromomethyl)cyclopropane,[23] 3,3′-bis(bromomethyl)biphenyl,[24] 4,4′-bis(bromomethyl)biphenyl,[25,26] 4-piperidinopyridine,[16] 4-(*N*-methylanilino)pyridine,[17] 4-(4-chloro-*N*-methylanilino)piridine,[18] 4-(3,5-dichloro-*N*-methylanilino)piridine,[18] **6**,[18] **7**,[18] and **8**[18] were synthesized according to literature procedures. 4-(Pyrrolidino)pyridine, 1,3-bis(bromomethyl)benzene, and 1,4-bis(bromomethyl)benzene were purchased from Aldrich.

General Experimental Procedure for the Preparation of Bispyridinium Compounds. A solution of the linker [bis(bromomethyl) compound] and the corresponding 4-substituted pyridine (in a 1:2 molar ratio) was heated at 100 °C in a sealed tube for a period of time that went from 15 and 192 h. After filtration and thorough washing with butanone, ethyl acetate, and diethyl ether, the solid was purified by recrystallization from ethanol or methanol after adding diethyl ether to turbidity.

1,1′-(*trans*-Cyclopropane-1,2-diylmethylene)bis[4-(pyrrolidino)pyridinium] Dibromide (1). Yield: 49.1% Mp 257 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.18 (d, J = 7.6, 4H, H-2,6$_{py}$); 6.83 (d, J = 7.6, 4H, H-3,5$_{py}$); 4.19 (dd, J_{gem} = 14.1, J_{vic} = 6.3, 2H, CH_2N^+); 4.02 (dd, J_{gem} = 14.1, J_{vic} = 7.4, 2H, CH_2N^+); 3.55 (m, J = 6.8, 8H, H-2,5$_{pyrrolid}$); 2.13 (q, J = 6.8, 8H, H-3,4$_{pyrrolid}$) 1.65 (m, J = 6.8, 2H, H-1,2$_{cyclopr}$); 0.97 (t, J = 6.8, 2H, H-3$_{cyclopr}$). ^{13}C NMR (75 MHz, CD_3OD) δ 157.96 (C-4$_{py}$); 142.69 (C-2,6$_{py}$); 109.54 (C-3,5$_{py}$); 61.09 (CH_2N^+); 49.64 (C-2,5$_{pyrrolid}$); 26.04 (C-3,4$_{pyrrolid}$); 19.68 (C-1,2$_{cyclopr}$); 10.48 (C-3$_{cyclopr}$). HR LSIMS (*m*/*z*) calcd for $C_{23}H_{32}N_4Br_2Na$ (M + Na)$^+$ 545.0891; found 545.0889. Anal. ($C_{23}H_{32}N_4Br_2\cdot1.2H_2O$) C, H, N.

1,1′-(*trans*-Cyclopropane-1,2-diylmethylene)bis[4-(piperidino)pyridinium] Dibromide (2). Yield: 47.6%. Mp 253 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (d, J = 7.9, 4H, H-2,6$_{py}$); 7.10 (d, J = 7.9, 4H, H-3,5$_{py}$); 4.16 (dd, J_{gem} = 14.1, J_{vic} = 6.4, 2H, CH_2N^+); 4.01 (dd, J_{gem} = 14.1, J_{vic} = 7.5, 2H, CH_2N^+); 3.70 (t, J = 5.5, 8H, H-2,6$_{pip}$); 1.69 (m, J = 5.5, 12H, H-3,4,5$_{pip}$) 1.63 (m, J = 6.8, 2H, H-1,2$_{cyclopr}$); 0.95 (t, J = 6.8, 2H, H-3$_{cyclopr}$). ^{13}C NMR (100 MHz, CD_3OD) δ 156.87 (C-4$_{py}$); 143.39 (C-2,6$_{py}$); 109.31 (C-3,5$_{py}$); 61.04 (CH_2N^+); 49.21 (C-2,6$_{pip}$); 26.70 (C-3,5$_{pip}$); 24.98 (C-4$_{pip}$); 19.69 (C-1,2$_{cyclopr}$); 10.61 (C-3$_{cyclopr}$). HR LSIMS (*m*/*z*) calcd for $C_{25}H_{36}N_4Br_2Na$ (M + Na)$^+$ 573.12204; found 573.1206. Anal. ($C_{25}H_{36}N_4Br_2\cdot1.38H_2O\cdot0.5CH_3CH_2OH$) C, H, N.

1,1′-(*trans*-Cyclopropane-1,2-diylmethylene)bis[4-(*N*-methylanilino)piridinium] Dibromide (3). Yield: 35.9%. Mp 52 °C (gel) − 220 °C (fusion). ^{1}H NMR (300 MHz, CD_3OD) δ 8.28 (bs, 4H, H-2,6$_{py}$); 7.61 (t, J = 7.4, 4H, H-3,5$_{anil}$); 7.51 (t, J = 7.4, 2H, H-4$_{anil}$); 7.36 (d, J = 7.4, 4H, H-2,6$_{anil}$); 6.90 (bs, 4H, H-3,5$_{py}$); 4.24 (dd, J_{gem} = 14.1, J_{vic} = 6.7, 2H, CH_2N^+); 4.11 (dd, J_{gem} = 14.1, J_{vic} = 6.9, 2H, CH_2N^+); 3.56 (s, 6H, CH_3); 1.66 (m, J = 6.7, 2H, H-1,2$_{cyclopr}$); 0.95 (t, J = 6.7, 2H, H-3$_{cyclopr}$). ^{13}C NMR (75 MHz, CD_3OD) δ 158.43 (C-4$_{py}$); 144.83 (C-1$_{anil}$); 143.57 (C-2,6$_{py}$); 132.04 (C-3,5$_{anil}$); 130.13 (C-4$_{anil}$); 127.52 (C-2,6$_{anil}$); 110.21 (C-3,5$_{py}$); 61.47 (CH_2N^+); 41.44 (CH_3); 19.61 (C-1,2$_{cyclopr}$); 10.53 (C-3$_{cyclopr}$). HR LSIMS (*m*/*z*) calcd for $C_{29}H_{32}N_4Br$ (M − Br)$^+$ 515.1810; found 515.1809. Anal. ($C_{39}H_{32}N_4Br_2\cdot2.5H_2O$) C, H, N.

1,1′-(*trans*-Cyclopropane-1,2-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)pyridinium] Dibromide (4). Yield: 62.4%. Mp 81 °C (gel) − 228 °C(fusion). ^{1}H NMR (300 MHz, CD_3OD) δ 8.31 (d, J = 7.1, 4H, H-2,6$_{py}$); 7.61 (t, J = 8.7, 4H, H-3,5$_{anil}$); 7.38 (d, J = 8.7, 4H, H-2,6$_{anil}$); 6.95 (bs, 4H, H-3,5$_{py}$); 4.28 (dd, J_{gem} = 14.1, J_{vic} = 6.5, 2H, CH_2N^+); 4.11 (dd, J_{gem} = 14.1, J_{vic} = 7.1, 2H, CH_2N^+); 3.55 (s, 6H, CH_3); 1.66 (m, J = 6.7, 2H, H-1,2$_{cyclopr}$); 0.96 (t, J = 6.7, 2H, H-3$_{cyclopr}$). ^{13}C NMR (75 MHz, CD_3OD) δ 158.45 (C-4$_{py}$); 143.70 (C-1$_{anil}$); 143.45 (C-2,6$_{py}$); 135.71(C-4$_{anil}$); 132.20 (C-3,5$_{anil}$); 129.40 (C-2,6$_{anil}$); 110.41 (C-3,5$_{py}$); 61.54 (CH_2N^+); 41.39 (CH_3); 19.59 (C-1,2$_{cyclopr}$); 10.54 (C-3$_{cyclopr}$). HR LSIMS (*m*/*z*) calcd for $C_{29}H_{30}N_4Cl_2Br$ (M − Br)$^+$ 583.1031; found 583.1026. Anal. ($C_{29}H_{30}N_4Br_2Cl_2\cdot H_2O$) C, H, N.

1,1′-(*trans*-Cyclopropane-1,2-diylmethylene)bis[4-(3,5-dichloro-*N*-methylanilino)pyridinium] Dibromide (5). Yield: 70.7%. Mp 174 °C (gel) − 256 °C (fusion). ^{1}H NMR (300 MHz, CD_3OD) δ 8.37 (d, J = 7.4, 4H, H-2,6$_{py}$); 7.63 (t, J = 1.7, 2H, H-4$_{anil}$); 7.49 (d, J = 1.7, 4H, H-2,6$_{anil}$); 7.00 (d, J = 7.4, 4H, H-3,5$_{py}$); 4.33 (dd, J_{gem} = 14.1, J_{vic} = 6.4, 2H, CH_2N^+); 4.13 (dd, J_{gem} = 14.1, J_{vic} = 7.1, 2H, CH_2N^+); 3.55 (s, 6H, CH_3); 1.69 (m, J = 6.7, 2H, H-1,2$_{cyclopr}$); 0.97 (t, J = 6.7, 2H, H-3$_{cyclopr}$). ^{13}C NMR (75 MHz, CD_3OD) δ 158.39 (C-4$_{py}$); 146.79 (C-1$_{anil}$); 143.97 (C-2,6$_{py}$); 138.01 (C-3,5$_{anil}$); 130.22 (C-4$_{anil}$); 127.03 (C-2,6$_{anil}$); 110.74 (C-3,5$_{py}$); 61.68 (CH_2N^+); 41.35 (CH_3); 19.63 (C-1,2$_{cyclopr}$); 10.58 (C-3$_{cyclopr}$). HR LSIMS (*m*/*z*) calcd for $C_{29}H_{28}N_4Cl_4Br$ (M − Br)$^+$ 651.0251; found 651.0248. Anal. ($C_{29}H_{28}N_4Br_2Cl_4\cdot2.5H_2O$) C, H, N.

1,1′-(Benzene-1,3-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)pyridinium] Dibromide (9). Yield: 62.7%. Mp 78.7 °C (gel) − 197 °C (fusion). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 6.6, 4H, H-2,6$_{py}$); 7.64 (d, J = 8.6, 4H, H-3,5$_{anil}$); 7.57 (s, 1H, H-2$_{ph}$); 7.45 (d, J = 8.6, 5H, H-2,6$_{anil}$ and H-5$_{ph}$); 7.37 (d, J = 7.7, 2H, H-4,6$_{ph}$); 6.95 (bs, 4H, H-3,5$_{py}$); 5.49 (s, 4H, CH_2N^+); 3.46 (s, 6H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.20 (C-4$_{py}$); 142.75 (C-2,6$_{py}$); 141.96 (C-1$_{anil}$); 136.18 (C-1,3$_{ph}$); 132.78 (C-4$_{anil}$); 130.50 (C-3,5$_{anil}$); 129.73 (C-5$_{ph}$); 128.37 (C-2,6$_{anil}$); 128.18 (C-4,6$_{ph}$); 127.89 (C-2$_{ph}$); 109.15 (C-3,5$_{py}$); 59.16 (CH_2N^+); 41.42 (CH_3). HR LSIMS (*m*/*z*) calcd for $C_{32}H_{30}N_4Cl_2Br$ (M − Br)$^+$ 619.1031; found 619.1031. Anal. ($C_{32}H_{30}N_4Cl_2Br_2\cdot1H_2O$) C, H, N.

1,1′-(Benzene-1,3-diylmethylene)bis[4-(3,5-dichloro-*N*-methylanilino)pyridinium] Dibromide (10). Yield: 75.3%. Mp 281 °C (gel) − 306 °C (fusion). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, J = 7.2, 4H, H-2,6$_{py}$); 7.75 (t, J = 1.5, 2H, H-4$_{anil}$); 7.64 (d, J = 1.5, 4H, H-2,6$_{anil}$); 7.60 (s, 1H, H-2$_{ph}$); 7.47−7.38 (m, 3H, H-4,5,6$_{ph}$); 7.05 (d, J = 7.2, 4H, H-3,5$_{py}$); 5.53 (s, 4H, CH_2N^+); 3.45 (s, 6H, CH_3).^{13}C NMR (75 MHz, DMSO-d_6) δ 156.25 (C-4$_{py}$); 145.27 (C-1$_{anil}$); 142.94 (C-2,6$_{py}$); 136.23 (C-1,3$_{ph}$); 135.36 (C-3,5$_{anil}$); 129.75 (C-5$_{ph}$); 128.36 y 128.28 (C-4,6$_{ph}$ and C-4$_{anil}$); 127.97 (C-2$_{ph}$); 125.97 (C-2,6$_{anil}$); 109.55 (C-3,5$_{py}$); 59.23 (CH_2N^+); 40.67 (CH_3). HR LSIMS (*m*/*z*): calcd for $C_{32}H_{28}N_4Cl_4Br$ (M − Br)$^+$ 687.0251; found 687.0249. Anal. ($C_{32}H_{28}N_4Cl_4Br_2$) C, H, N.

1,1′-(Benzene-1,4-diylmethylene)bis[4-(pyrrolidino)pyridinium] Dibromide (11). Yield: 63.2%. Mp 324 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.21 (d, J = 7.6, 4H, H-2,6$_{py}$); 7.46 (s, 4H, H-2,3,5,6$_{ph}$); 6.86 (d, J = 7.6, 4H, H-3,5$_{py}$); 5.39 (s, 4H, CH_2N^+); 3.55 (t, J = 6.8, 8H, H-2,5$_{pyrrolid}$); 2.11 (q, J = 6.8, 8H, H-3,4$_{pyrrolid}$). ^{13}C NMR (75 MHz, CD_3OD) δ 155.17 (C-4$_{py}$); 143.07 (C-2,6$_{py}$); 137.38 (C-1,4$_{ph}$); 130.24 (C-2,3,5,6$_{ph}$); 109.79 (C-3,5$_{py}$); 61.13 (CH_2N^+); 49.77 (C-2,5$_{pyrrolid}$); 26.12 (C-3,4$_{pyrrolid}$). HR LSIMS (*m*/*z*) calcd for $C_{26}H_{32}N_4Br$ (M − Br)$^+$ 479.1810; found 479.1815. Anal. ($C_{26}H_{32}N_4Br_2\cdot 2.5H_2O$) C, H, N.

1,1′-(Benzene-1,4-diylmethylene)bis[4-(piperidino)pyridinium] Dibromide (12). Yield: 79.6%. Mp 316 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.20 (d, J = 7.8, 4H, H-2,6$_{py}$); 7.47 (s, 4H, H-2,3,5,6$_{ph}$); 7.14 (d, J = 7.8, 4H, H-3,5$_{py}$); 5.37 (s, 4H, CH_2N^+); 3.70 (t, J = 5.3, 8H, H-2,6$_{pip}$); 1.71 (m, J = 5.3, 12H, H-3,4,5$_{pip}$). ^{13}C NMR (75 MHz, CD_3OD) δ 156.83 (C-4$_{py}$); 143.61 (C-2,6$_{py}$); 137.28 (C-1,4$_{ph}$); 130.32 (C-2,3,5,6$_{ph}$); 109.36 (C-3,5$_{py}$); 60.96 (CH_2N^+); 49.14 (C-2,6$_{pip}$); 26.71 (C-3,5$_{pip}$); 24.94 (C-4$_{pip}$). HR LSIMS (*m*/*z*) calcd for $C_{28}H_{36}N_4Br$ (M − Br)$^+$ 507.2123; found 507.2118. Anal. ($C_{28}H_{36}N_4Br_2\cdot 0.5H_2O$) C, H, N.

1,1′-(Benzene-1,4-diylmethylene)bis[4-(*N*-methylanilino)pyridinium] Dibromide (13). Yield: 71.9%. Mp 349.5 °C.^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (bs, 4H, H-2,6$_{py}$); 7.60 (t, 4H, H-3,5$_{anil}$, J = 7.4); 7.51 (m, 4H, H-4$_{anil}$, J = 7.4); 7.47 (s, 4H, H-2,3,5,6$_{ph}$); 7.35 (d, 4H, H-2,6$_{anil}$, J = 7.4); 6.99 (bs, 4H, H-3,5$_{py}$); 5.43 (s, 4H, CH_2N^+); 3.54 (s, 6H, CH_3). ^{13}C NMR (75 MHz, CD_3OD) δ 158.39 (C-4$_{py}$); 144.62 (C-1$_{anil}$); 143.70 (C-2,6$_{py}$); 137.04 (C-1,4$_{ph}$); 131.92 (C-3,5$_{anil}$); 130.25 y 130.08 (C-2,3,5,6$_{ph}$ and C-4$_{anil}$); 127.32 (C-2,6$_{anil}$); 110.18 (C-3,5$_{py}$); 61.31 (CH_2N^+); 41.27 (CH_3). HR LSIMS (*m*/*z*) calcd for $C_{32}H_{32}N_4Br$ (M − Br)$^+$ 551.1810; found 551.1812. Anal. ($C_{32}H_{32}N_4Br_2\cdot 0.75H_2O$) C, H, N.

1,1′-(Benzene-1,4-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)pyridinium] Dibromide (14). Yield: 55.6%. Mp 332 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (d, J = 6.2, 4H, H-2,6$_{py}$); 7.63 (d, J = 8.5, 4H, H-3,5$_{anil}$); 7.46 (s, 4H, H-2,3,5,6$_{ph}$); 7.43 (d, J = 8.5, 4H, H-2,6$_{anil}$); 6.91 (bs, 4H, H-3,5$_{py}$); 5.47 (s, 4H, CH_2N^+); 3.43 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.21 (C-4$_{py}$); 142.73 (C-1$_{anil}$); 142.03 (C-2,6$_{py}$); 136.06 (C-1,4$_{ph}$); 132.82 (C-4$_{anil}$); 130.55 (C-3,5$_{anil}$); 128.74 (C-2,3,5,6$_{ph}$); 128.45 (C-2,6$_{anil}$); 109.22 (C-3,5$_{py}$); 59.03 (CH_2N^+); 40.76 (CH_3). HR LSIMS (*m*/*z*) calcd for $C_{32}H_{30}N_4Cl_2Br$ (M − Br)$^+$ 619.1031; found 619.1031. Anal. ($C_{32}H_{30}N_4Cl_2Br_2\cdot 0.1H_2O$) C, H, N.

1,1′-(Benzene-1,4-diylmethylene)bis[4-(3,5-dichloro-*N*-methylanilino)pyridinium] Dibromide (15). Yield: 63%. Mp 314.7 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (d, J = 7.3, 4H, H-2,6$_{py}$); 7.75 (t, J = 1.7, 2H, H-4$_{anil}$); 7.61 (d, J = 1.7, 4H, H-2,6$_{anil}$); 7.48 (s, 4H, H-2,3,5,6$_{ph}$); 7.21 (d, J = 7.3, 4H, H-3,5$_{py}$); 5.53 (s, 4H, CH_2N^+); 3.43 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.23 (C-4$_{py}$); 145.25 (C-1$_{anil}$); 142.85 (C-2,6$_{py}$); 136.02 (C-1,4$_{ph}$); 135.36 (C-3,5$_{anil}$); 128.77 (C-2,3,5,6$_{ph}$); 128.37 (C-4$_{anil}$); 125.97 (C-2,6$_{anil}$); 109.55 (C-3,5$_{py}$); 59.13 (CH_2N^+); 40.62 (CH_3). HR LSIMS (*m*/*z*) calcd for $C_{32}H_{28}N_4Cl_4Br$ (M − Br)$^+$ 687.0251; found 687.0252. Anal. ($C_{32}H_{28}N_4Cl_4Br_2\cdot H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(pyrrolidino)pyridinium] Dibromide (16). Yield: 87.4%. Mp 115 °C (gel) − 187 °C (fusion). ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (d, J = 7.7, 4H, H-2,6$_{py}$); 7.83 (s, 2H, H-2$_{ph}$); 7.70 (d, J = 7.7, 2H, H-6$_{ph}$); 7.54 (t, J = 7.7, 2H, H-5$_{ph}$); 7.42 (d, J = 7.7, 2H, H-4$_{ph}$); 6.89 (d, J = 7.7, 4H, H-3,5$_{py}$); 5.47 (s, 4H, CH_2N^+); 3.58 (t, J = 6.8, 8H, H-2,5$_{pyrrolid}$); 2.13 (q, J = 6.8, 8H, H-3,4$_{pyrrolid}$). ^{13}C NMR (100 MHz, CD_3OD) δ 155.17 (C-4$_{py}$); 143.08 (C-2,6$_{py}$); 142.62 (C-1$_{ph}$); 137.15 (C-3$_{ph}$); 131.09 (C-5$_{ph}$); 128.87 (C-4$_{ph}$); 128.66 (C-6$_{ph}$); 128.37 (C-2$_{ph}$); 109.78 (C-3,5$_{py}$); 61.63 (CH_2N^+); 49.72 (C-2,5$_{pyrrolid}$); 26.12 (C-3,4$_{pyrrolid}$). HR LSIMS (*m*/*z*) calcd for $C_{32}H_{36}N_4Br$ (M − Br)$^+$ 555.2123; found 555.2121. Anal. ($C_{32}H_{36}N_4Br_2\cdot 0.3H_2O\cdot 1.5CH_3CH_2OH$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(piperidino)pyridinium] Dibromide (17). Yield: 86.2%. Mp 269 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.31 (d, J = 7.7, 4H, H-2,6$_{py}$); 7.85 (s, 2H, H-2$_{ph}$); 7.69 (d, J = 7.7, 2H, H-6$_{ph}$); 7.52 (t, J = 7.7, 2H, H-5$_{ph}$); 7.41 (d, J = 7.7, 2H, H-4$_{ph}$); 7.18 (d, J = 7.7, 4H, H-3,5$_{py}$); 5.44 (s, 4H, CH_2N^+); 3.70 (t, J = 5.5, 8H, H-2,6$_{pip}$); 1.69 (m, 12H, H-3,4,5$_{pip}$).^{13}C NMR (75 MHz, CD_3OD) δ 156.84 (C-4$_{py}$); 143.61 (C-2,6$_{py}$); 142.60 (C-1$_{ph}$); 137.02 (C-3$_{ph}$); 131.12 (C-5$_{ph}$); 128.90 (C-4$_{ph}$); 128.76 (C-6$_{ph}$); 128.51 (C-2$_{ph}$); 109.36 (C-3,5$_{py}$); 61.47 (CH_2N^+); 49.11 (C-2,6$_{pip}$); 26.68 (C-3,5$_{pip}$); 24.93 (C-4$_{pip}$). HR LSIMS (*m*/*z*) calcd for $C_{34}H_{40}N_4Br$ (M − Br)$^+$ 583.2440; found 583.2440. Anal. ($C_{34}H_{40}N_4Br_2\cdot H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(*N*-methylanilino)pyridinium] Dibromide (18). Yield: 71.5%. Mp. 124 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.35 (bs, 4H, H-2,6$_{py}$); 7.84 (s, 2H, H-2$_{ph}$); 7.67 (d, J = 7.7, 2H, H-6$_{ph}$); 7.56 (t, J = 7.6, 4H, H-3,5$_{anil}$); 7.50−7.44 (m, 4H, H-5$_{ph}$ and H-4$_{anil}$); 7.39 (d, J = 7.7, 2H, H-4$_{ph}$); 7.33 (d, J = 7.5, 4H, H-2,6$_{anil}$); 6.95 (bs, 4H, H-3,5$_{py}$); 5.47 (s, 4H, CH_2N^+); 3.51 (s, 6H, CH_3). ^{13}C NMR (75 MHz, CD_3OD) δ 158.48 (C-4$_{py}$); 144.82 (C-1$_{anil}$); 143.80 (C-2,6$_{py}$); 142.60 (C-1$_{ph}$); 136.82 (C-3$_{ph}$); 132.01 (C-3,5$_{anil}$); 131.14 (C-5$_{ph}$); 130.12 (C-4$_{anil}$); 128.99 (C-4$_{ph}$); 128.82 (C-6$_{ph}$); 128.58 (C-2$_{ph}$); 127.52 (C-2,6$_{anil}$); 110.29 (C-3,5$_{py}$); 61.97 (CH_2N^+); 41.42 (CH_3). HR LSIMS (*m*/*z*) calcd for $C_{38}H_{36}N_4Br$ (M − Br)$^+$ 627.2123; found 627.2122. Anal. ($C_{38}H_{36}N_4Br_2\cdot 2.5H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)pyridinium] Dibromide (19). Yiled: 49.7%. Mp 231 °C (gel) − 279 °C (fusion). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (d, J = 6.5, 4H, H-2,6$_{py}$); 7.88 (s, 2H, H-2$_{ph}$); 7.67 (d, J = 7.7, 2H, H-6$_{ph}$); 7.61 (d, J = 8.6, 4H, H-3,5$_{anil}$); 7.51 (t, J = 7.7, 2H, H-5$_{ph}$); 7.42 (d, J = 8.6, 6H, H-4$_{ph}$ and H-2,6$_{anil}$); 6.99 (bs, 4H, H-3,5$_{py}$); 5.51 (s, 4H, CH_2N^+); 3.43 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.20 (C-4$_{py}$); 142.72 (C-2,6$_{py}$); 142.05 (C-1$_{anil}$); 140.01 (C-1$_{ph}$); 136.20 (C-3$_{ph}$); 132.79 (C-4$_{anil}$); 130.53 (C-3,5$_{anil}$); 129.73 (C-5$_{ph}$); 128.47 (C-2,6$_{anil}$); 127.51 (C-4$_{ph}$); 127.14 (C-6$_{ph}$); 127.04 (C-2$_{ph}$); 109.20 (C-3,5$_{py}$); 59.55 (CH_2N^+); 40.73 (CH_3). HR LSIMS (*m*/*z*) calcd for $C_{38}H_{34}N_4Cl_2Br$ (M − Br)$^+$ 695.1344; found 695.1344. Anal. ($C_{38}H_{34}N_4Cl_2Br_2\cdot 1.2H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(3,5-dichloro-*N*-methylanilino)pyridinium] Dibromide (20). Yield: 79.7%. Mp 312 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (d, J = 7.1, 4H, H-2,6$_{py}$); 7.92 (s, 2H, H-2$_{ph}$); 7.75 (s, 2H, H-4$_{anil}$); 7.70 (d, J = 7.6, 2H, H-6$_{ph}$); 7.62 (d, J = 1.8, 4H, H-2,6$_{anil}$); 7.53 (t, J = 7.6, 2H, H-5$_{ph}$); 7.45 (d, J = 7.6, 2H, H-4$_{ph}$); 7.04 (d, J = 7.1, 4H, H-3,5$_{py}$); 5.56 (s, 4H, CH_2N^+); 3.44 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.20 (C-4$_{py}$); 145.27 (C-1$_{anil}$); 142.86 (C-2,6$_{py}$); 140.08 (C-1$_{ph}$); 136.11 (C-3$_{ph}$); 135.34 (C-3,5$_{anil}$); 129.70 (C-5$_{ph}$); 128.33 (C-4$_{anil}$); 127.55 (C-4$_{ph}$); 127.14 (C-6$_{ph}$); 127.07 (C-2$_{ph}$); 125.97 (C-2,6$_{anil}$); 109.53 (C-3,5$_{py}$); 59.65 (CH_2N^+); 40.59 (CH_3). HR LSIMS (*m*/*z*) calcd for $C_{38}H_{32}N_4Cl_4Br$ (M − Br)$^+$ 763.0564; found 763.0561. Anal. ($C_{38}H_{32}N_4Cl_4Br_2\cdot 0.1H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(pyrrolidino)pyridinium] Dibromide (21). Yield: 36.5%. Mp 202 °C (gel) − 331 °C (fusion). ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (d, J = 7.7, 4H, H-2,6$_{py}$); 7.71 (d, J = 8.2, 4H, H-2,6$_{ph}$); 7.51 (d, J = 8.2, 4H, H-3,5$_{ph}$); 6.90 (d, J = 7.7, 4H, H-3,5$_{py}$); 5.44 (s, 4H, CH_2N^+); 3.58 (t, J = 6.8, 8H, H-2,5$_{pyrrolid}$); 2.13 (q, J = 6.8, 8H, H-3,4$_{pyrrolid}$). ^{13}C NMR (100 MHz, CD_3OD) δ 155.19 (C-4$_{py}$); 143.06 (C-2,6$_{py}$); 142.22 (C-1$_{ph}$); 135.79 (C-4$_{ph}$); 130.01 (C-3,5$_{ph}$); 128.76(C-2,6$_{ph}$); 109.76 (C-3,5$_{py}$); 61.40 (CH_2N^+); 49.75 (C-2,5$_{pyrrolid}$); 26.12 (C-3,4$_{pyrrolid}$). HR LSIMS (*m*/*z*) calcd for $C_{32}H_{36}N_4Br$ (M + Br)$^+$ 555.2123; found 555.2130. Anal. ($C_{32}H_{36}N_4Br_2\cdot 0.5H_2O\cdot 0.9CH_3CH_2OH$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(piperidino)pyridinium] Dibromide (22). Yield: 33.7%. Mp 281 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (d, J = 7.9, 4H, H-2,6$_{py}$); 7.69 (d, J = 8.3, 4H, H-2,6$_{ph}$); 7.49 (d, J = 8.3, 2H, H-3,5$_{ph}$); 7.14 (d, J = 7.9, 4H, H-3,5$_{py}$); 5.39 (s, 4H, CH_2N^+); 3.70 (t, J = 5.5, 8H, H-2,6$_{pip}$); 1.73 (m, 12H, H-3,4,5$_{pip}$). ^{13}C NMR (100 MHz, CD_3OD) δ 156.86 (C-4$_{py}$); 143.58 (C-2,6$_{py}$); 142.26 (C-1$_{ph}$); 135.68 (C-4$_{ph}$); 130.05 (C-3,5$_{ph}$); 128.94 (C-2,6$_{ph}$); 109.32 (C-3,5$_{py}$); 61.47 (CH_2N^+); 49.11 (C-2,6$_{pip}$); 26.68 (C-3,5$_{pip}$); 24.93 (C-4$_{pip}$). HR LSIMS (m/z) calcd for $C_{34}H_{40}N_4Br$ $(M - Br)^+$ 583.2436; found 583.2433. Anal. ($C_{34}H_{40}N_4Br_2 \cdot 1H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(*N*-methylanilino)pyridinium] Dibromide (23). Yield: 74.5%. Mp 85 °C (gel) − 205 °C(fusion). ^{1}H NMR (300 MHz, CD_3OD) δ 8.35 (bs, 4H, H-2,6$_{py}$); 7.70 (d, J = 8.2, 4H, H-2,6$_{ph}$); 7.60 (t, J = 7.6, 4H, H-3,5$_{anil}$); 7.51 (d, J = 7.6, 6H, H-3,5$_{ph}$ and H-4$_{anil}$); 7.37 (d, J = 7.6, 4H, H-2,6$_{anil}$); 6.95 (bs, 4H, H-3,5$_{py}$); 5.47 (s, 4H, CH_2N^+); 3.55 (s, 6H, CH_3). ^{13}C NMR (100 MHz, CD_3OD) δ 158.47 (C-4$_{py}$); 144.79 (C-1$_{anil}$); 143.76 (C-2,6$_{py}$); 142.30 (C-1$_{ph}$); 135.51 (C-4$_{ph}$); 132.01 (C-3,5$_{anil}$); 130.13 (C-3,5$_{ph}$ and C-4$_{anil}$); 128.96 (C-2,6$_{ph}$); 127.49 (C-2,6$_{anil}$); 110.25 (C-3,5$_{py}$); 61.70 (CH_2N^+); 41.43 (CH_3). HR LSIMS (m/z) calcd for $C_{38}H_{36}N_4Br$ $(M - Br)^+$ 627.2123; found 627.2123. Anal. ($C_{38}H_{36}N_4Br_2 \cdot 3H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)pyridinium] Dibromide (24). Yield: 90.1%. Mp 120 °C (gel) − 217 °C (fusion). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (d, J = 7.6, 4H, H-2,6$_{py}$); 7.69 (d, J = 8.2, 4H, H-2,6$_{ph}$); 7.63 (t, J = 8.6, 4H, H-3,5$_{anil}$); 7.51 (d, J = 8.2, 4H, H-3,5$_{ph}$); 7.44 (d, J = 8.6, 4H, H-2,6$_{anil}$); 6.99 (bs, 4H, H-3,5$_{py}$); 5.50 (s, 4H, CH_2N^+); 3.44 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.23 (C-4$_{py}$); 142.75 (C-2,6$_{py}$); 142.08 (C-1$_{anil}$); 139.66 (C-1$_{ph}$); 134.97 (C-4$_{ph}$); 132.81 (C-4$_{anil}$); 130.56 (C-3,5$_{anil}$); 128.73 (C-3,5$_{ph}$); 128.48 (C-2,6$_{anil}$); 127.30 (C-2,6$_{ph}$); 109.24 (C-3,5$_{py}$); 59.22 (CH_2N^+); 40.74 (CH_3). HR LSIMS (m/z) calcd for $C_{38}H_{34}N_4Cl_2Br$ $(M - Br)^+$ 695.1344; found 695.1342. Anal. ($C_{38}H_{34}N_4Cl_2Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(3,5-dichloro-*N*-methylanilino)pyridinium] Dibromide (25). Yield: 69.5%. Mp 313 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (d, J = 7.2, 4H, H-2,6$_{py}$); 7.77 (t, J = 1.6, 2H, H-4$_{anil}$); 7.71 (d, J = 8.2, 4H, H-2,6$_{ph}$); 7.62 (d, J = 1.6, 4H, H-2,6$_{anil}$); 7.52 (d, J = 8.2, 4H, H-3,5$_{ph}$); 7.05 (d, J = 7.2, 4H, H-3,5$_{py}$); 5.53 (s, 4H, CH_2N^+); 3.44 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.24 (C-4$_{py}$); 145.28 (C-1$_{anil}$); 142.88 (C-2,6$_{py}$); 139.69 (C-1$_{ph}$); 135.37 (C-3,5$_{anil}$); 134.88 (C-4$_{ph}$); 128.77 (C-3,5$_{ph}$); 128.35 (C-4$_{anil}$); 127.28 (C-2,6$_{ph}$); 125.98(C-2,6$_{anil}$); 109.57 (C-3,5$_{py}$); 59.33 (CH_2N^+); 40.60 (CH_3). HR LSIMS (m/z) calcd for $C_{38}H_{32}N_4Cl_4Br$ $(M - Br)^+$ 763.0564; found 763.0559. Anal: ($C_{38}H_{32}N_4Cl_4Br_2 \cdot H_2O$) C, H, N.

(b) Pharmacology. The ex vivo human ChoK inhibition and antiproliferative assays against HT-29 cells were followed in accordance with the protocols previously reported.[9,12,28] The results are recorded in Tables 1 and 2.

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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